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THE APPLICATION OF THE COMPLEMENT-FIXATION REACTION TO THE DIPHTHERIA GROUP OF ORGANISMS.*†

MARY ELIZABETH MORSE.

(From the Laboratory of Pathology [Phillips Fund], Harvard Medical School, Boston, Mass.)

The present work forms the second part of a study of the diphtheria group of organisms. The writer is greatly indebted to Dr. H. T. Karsner for advice and the use of the facilities of the experimental division of the laboratory.

The first part of the investigation consisted of a study of the group by the biometrical method.[1] It was found that the group could be divided into two main subgroups, the first comprising the diphtheria bacillus, the second the diphtheroids and Hofmann's bacillus. The second subgroup could be separated further into four species, which are characterized as follows:

B. hoagii, a short, thick bacillus, solid, barred, or wedge-shaped, with indistinct granules; growing very heavily on serum with a salmon-pink color, and fermenting dextrose and saccharose, but not maltose or glycerin.

B. flavidus, a thick, barred bacillus with large granules; having a heavy yellow or yellow-white growth on serum, and acting on dextrose, glycerin, and maltose, but not on saccharose.

B. xerosis, resembling *B. flavidus* morphologically, but giving a meager white growth on serum. It acidifies dextrose, and usually both maltose and saccharose.

B. hofmanni, a small, thick, straight bacillus, barred or wedge-shaped, having no granules, and not acting upon dextrose, maltose, glycerin, or saccharose.

Complement fixation in the diphtheria group has been little studied. Lambotte[2] produced strong specific sera in guinea-pigs for the diphtheria bacillus, a "pseudo-diphtheria" bacillus from the

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*Received for publication August 3, 1912.

† Aided by a grant from the Rockefeller Institute for Medical Research.

[1] *Jour. Infect. Dis.*, 1912, 11, p. 253.

[2] *Centralbl. f. Bakt.*, 1901, 30, p. 817.

normal human conjunctiva, and also one from a membrane on a fowl's eye. He gives no description of these bacilli in the abovementioned article. He found that both the anti-diphtheroid sera gave partial fixation with the diphtheria bacillus.

Weill-Halle and Bloch-Michel[1] used as antigen a suspension of mucus from the throat in salt solution, and anti-diphtheritic serum as antibody. Their results were positive in 25 cases of diphtheria (controlled by culture), and negative in 10 cases of nondiphtheric angina, nine cases of measles, and three of scarlatina. The authors recommend the complement-fixation reaction as quicker and surer than the microscopic diagnosis, for the early detection of diphtheria.

Priestley[2] attempted to use the complement-fixation reaction for the differentiation of the diphtheria bacillus from the diphtheroids, but could obtain no results because of the lack of satisfactory antigens, although he used both bacterial extracts and bacterial solutions in weak antiformin.

Kolmer,[3] using bacterial extracts, found reciprocal reactions among different strains of diphtheria bacilli and also between the diphtheria bacillus and the Hofmann organism.

Some work has also been done on the fixation of complement using diphtheria toxin as antigen. Armand-Delille,[4] employing this with anti-diphtheria horse serum, obtained fixation. He found that there were great individual differences in the fixing power of the sera, and that the horses whose sera deviated strongly had shown signs of anaphylaxis during immunization, while those animals whose sera did not deviate had had no "accidents" during treatment. The author also found that there was no relation between the antitoxic and the fixing properties of the sera.

These results were confirmed by Poujol and Delanoe.[5] Cathoire[6] also found that the serum of healthy diphtheria carriers deviated complement in the presence of diphtheria toxin.

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[1] *Soc. méd. d. Hôp. de Paris*, 1910, 29, p. 707.

[2] *Proc. Royal Soc. of Med.*, 1911, 5, p. 146.

[3] *Jour. Infect. Dis.*, 1912, 11, p. 44.

[4] *Mém. de la Soc. de Biol.*, 1908, 65, p. 417.

[5] *Ibid.*, 1908, 67, p. 614.

[6] *Compt. rend. Soc. de Biol.*, 1911, 71, p. 315.

In the present experiments the following representative cultures were used for antigens. For the diphtheria bacillus, Park No. 8, the standard antitoxin strain; for *B. hoagii* a strain obtained from Dr. Louis Hoag, and isolated by him from sputum;[1] for *B. flavidus* a culture obtained by Dr. Hoag from the eye; for *B. xerosis* an organism isolated at the Hoagland Laboratory from a septic uterus; and for *B. hofmanni* a culture, "Cameron," obtained by Dr. Anna Williams of the Research Laboratories of the New York Board of Health. A culture "W," also sent by Dr. Williams, was used in addition. This strain, while showing involution forms similar to the Klebs-Loeffler bacillus, also presented characteristics of the Hoag bacillus, i.e., a slightly pink growth on serum, fermentation of saccharose, and absence of action on maltose and glycerin.

In some preliminary work formalized cultures of the bacilli were used as antigens. They were found to be unsatisfactory, however, on account of their marked anti-complementary properties, and bacillary extracts were therefore substituted for them. The method of preparation of these extracts was the same as that employed by Swift and Thoro,[2] with minor changes. The organisms were grown on serum slants in large bottles for four days, this length of time being necessary to secure a sufficient growth of *B. diphtheriae* and *B. xerosis*. The bacillary suspension was washed once. The organisms, after being dried over sulphuric acid, and weighed, were suspended in 10 c.c. of salt solution, containing 0.5 per cent carbolic acid; ground for one hour in an agate mortar with powdered quartz, and shaken for 18 hours in an International Instrument Company centrifuge shaker at about 500 oscillations per minute. A solution containing one milligram of bacterial substance to one cubic centimeter of salt solution was prepared as needed from the concentrated extract.

Specific sera were obtained by the intravenous inoculation of rabbits. Three animals were immunized for each strain, and the strongest serum was used for the experiments. Formalized suspensions of the organisms were used, from six to 10 doses being given at intervals of five to seven days. A number of animals succumbed to inoculations with the diphtheria bacillus, so that the treatment had to be conducted very cautiously, beginning with doses of 0.1 c.c. of the suspension. Potent antisera for *B. diphtheriae*, *B. hoagii*, and *B. flavidus* were produced in this way, but no results were obtained with *B. xerosis* and *B. hofmanni*. Immunization with extracts of these two strains was then undertaken, but proved to be equally unsuccessful. The present study is limited, therefore, to the reactions of the three sera, *B. hoagii*, *B. flavidus*, and *B. diphtheriae*, with six antigens.

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[1] *Boston Med. and Surg. Jour.*, 1907, 157, p. 10.

[2] *Arch. of Int. Med.*, 1911, 7, p. 24.

A rabbit anti-sheep hemolytic system and guinea-pig complement were used.

The method of procedure was in outline as follows:

1. Determination of the strength of the hemolytic serum. The complement was then titrated each day to the serum.
2. Determination of the anti-complementary properties of the antigens.
3. Determination of the fixing dose of each antigen with normal and immune serum.
4. Trial of each immune serum with descending amounts of each antigen, to discover cross-reactions.

The results of the reactions are given in Tables 1, 2, and 3.

From Table 1 it is seen that the serum *B. diphtheriae* gave partial fixation with the homologous antigen in an amount of 0.025 mg. but that it did not react with any of the diphtheroid antigens or with the atypical diphtheria strain "W."

The serum *B. flavidus* (Table 2) fixed completely with its antigen in amounts of 0.025 mg. It also reacted with *B. hoagii*, *B. xerosis*, and the strain "W" at 0.1 mg., and fixed partially with *B. hofmanni* at 0.4 mg. It did not react, however, with the antigen *B. diphtheriae*.

The serum *B. hoagii* fixed partially with its specific antigen in a dose of 0.025 mg. It also fixed with antigen *B. flavidus* and "W" in amounts of 0.05 mg., but did not react with *B. xerosis*, *B. hofmanni*, or *B. diphtheriae*.

TABLE 1.

FIXING POWERS OF ANTIGENS WITH SERUM *B. diphtheriae.*

ANTIGEN	IMMUNE SERUM *B. diphtheriae*	NORMAL SERUM	COMPLEMENT		HEMOLYTIC SERUM	CORPUSCLES 5 PER CENT SUSPENSION		RESULTS	
								Serum *B. diphtheriae*	Normal Serum
B. diphtheriae—									
0.05 mg..........	0.1 c.c.	0.1 c.c.	2 units	Incubated one-half hour at 37.5° C.	2 units	1 c.c.	Incubated one-half hour at 37.5° C.	Fixation	Hemolysis
0.025 mg.........	"	"	"		"	"		Partial fixation	"
B. hoagii—	"								
0.1 mg...........	"	"	"		"	"		Hemolysis	Slight fixation
0.05 mg..........		"	"		"	"		"	" "
B. flavidus—	"								
0.4 mg...........	"	"	"		"	"		Fixation	Fixation
0.2 mg...........	"	"	"		"	"		Slight fixation	Slight fixation
0.1 mg...........	"	"	"		"	"		Very slight fixation	Very slight fixation
0.05 mg..........		"	"		"	"		Hemolysis	Hemolysis
B. xerosis—	"								
0.4 mg...........	"	"	"		"	"		"	"
0.2 mg...........	"	"	"		"	"		"	"
0.1 mg...........		"	"		"	"		"	"
B. hofmanni—	"								
0.6 mg...........	"	"	"		"	"		"	"
0.4 mg...........		"	"		"	"		"	"
"W"—									
0.2 mg...........	"	"	"		"	"		Fixation	Fixation
0.1 mg...........	"	"	"		"	"		Hemolysis	Hemolysis
Controls—									
NaCl 1 c.c........	"	"	"		"	"		"	"
NaCl 1 c.c.......	"	"	....		....	"		No hemolysis	No hemolysis

TABLE 2.

FIXING POWERS OF ANTIGENS WITH SERUM *B. flavidus.*

ANTIGEN	IMMUNE SERUM *B. flavidus*	NORMAL SERUM	COMPLEMENT		HEMOLYTIC SERUM	CORPUSCLES 5 PER CENT SUSPENSION		RESULTS	
								Serum *B. flavidus*	Normal Serum
B. flavidus—									
0.4 mg...........	0.1 c.c.	0.1 c.c.	2 units	Incubated one-half hour at 37.5° C.	2 units	1 c.c.	Incubated one-half hour at 37.5° C.	Fixation	Fixation
0.2 mg...........	“	“	“		“	“		“	Slight fixation
0.1 mg...........	“	“	“		“	“		“	Very slight fixation
0.05 mg..........	“	“	“		“	“		“	Hemolysis
0.025 mg.........	“	“	“		“	“		“	“
B. diphtheriae—									
0.4 mg...........	“	“	“		“	“		Very slight fixation	Very slight fixation
0.2 mg...........	“	“	“		“	“		Hemolysis	“ “ “
B. hoagii—									
0.1 mg...........	“	“	“		“	“		Fixation	Slight fixation
0.05 mg..........	“	“	“		“	“		Hemolysis	Hemolysis
B. xerosis—									
0.4 mg...........	“	“	“		“	“		Fixation	“
0.2 mg...........	“	“	“		“	“		“	“
0.1 mg...........	“	“	“		“	“		“	“
B. hofmanni—									
0.6 mg...........	“	“	“		“	“		Partial fixation	“
0.4 mg...........	“	“	“		“	“		“ “	“
0.2 mg...........	“	“	“		“	“		Hemolysis	“
“W”—									
0.2 mg...........	“	“	“		“	“		Fixation	Partial fixation
0.1 mg...........	“	“	“		“	“		“	Slight fixation
0.05 mg..........	“	“	“		“	“		Hemolysis	Hemolysis
Controls— As in Table 1									

TABLE 3.

FIXING POWERS OF ANTIGENS WITH SERUM *B. hoagii.*

ANTIGEN	IMMUNE SERUM *B. hoagii*	NORMAL SERUM	COMPLEMENT		HEMOLYTIC SERUM	CORPUSCLES 5 PER CENT SUSPENSION		RESULTS	
								Serum *B. hoagii*	Normal Serum
B. hoagii—									
0.1 mg.	0.1 c.c.	0.1 c.c.	2 units	Incubated one-half hour at 37.5° C.	2 units	1 c.c.	Incubated one-half hour at 37.5° C.	Fixation	Partial fixation
0.0 5 mg.	"	"	"		"	"		Partial fixation	Slight fixation
0.025 mg.	"	"	"		"	"		" "	Hemolysis
B. diphtheriae									
0.2 mg.	"	"	"		"	"		Hemolysis	Very slight fixation
0.1 mg.	"	"	"		"	"		"	Hemolysis
B. flavidus—									
0.4 mg.	"	"	"		"	"		Fixation	Fixation
0.2 mg.	"	"	"		"	"		"	Slight fixation
0.1 mg.	"	"	"		"	"		Partial fixation	Hemolysis
0.05 mg.	"	"	"		"	"		" "	"
0.025 mg.	"	"	"		"	"		Hemolysis	"
B. xerosis—									
0.4 mg.	"	"	"		"	"		"	"
0.2 mg.	"	"	"		"	"		"	"
B. hofmanni—									
0.6 mg.	"	"	"		"	"		"	"
0.4 mg.	"	"	"		"	"		"	"
"W"—									
0.2 mg.	"	"	"		"	"		Fixation	Partial fixation
0.1 mg.	"	"	"		"	"		"	Slight fixation
0.05 mg.	"	"	"		"	"		Partial fixation	Hemolysis
Controls— As in Table 1									

CONCLUSIONS.

The conclusions to be drawn from these experiments are:

1. There are no reciprocal complement-fixation reactions between the typical diphtheria bacillus and representatives of the four species of the diphtheroid group.

2. There are cross-reactions, however, within the diphtheroid group. The species *B. hoagii*, *B. flavidus*, and *B. xerosis* are closely related serologically, as well as morphologically and culturally.

3. *B. hofmanni*, although differing entirely in fermentation powers from the diphtheroids, yet is proved by the complement-fixation test to be related to *B. flavidus*, and therefore must be placed definitely in the diphtheroid group.

4. The action of the culture "W," which is certainly closely allied, both morphologically and culturally, to the diphtheria bacillus, and yet reacts with both *B. hoagii* and *B. flavidus* sera, is further evidence that there are connections between the groups of the diphtheria bacillus and of the diphtheroids.